JHR 98: 339–353 (2025)
doi: 10.3897/jhr.98.138425
https://jhr.pensoft.net

RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

Apanteles piceotrichosus Blanchard (Hymenoptera, Braconidae), first record from México, new molecular and biological data

Susana E. Rodríguez-Rodríguez[1], José L. Fernández-Triana[2], J. Refugio Lomeli-Flores[1], Marcelino Martínez-Núñez[1], Esteban Rodríguez-Leyva[1], Héctor González-Hernández[1], Julio S. Bernal[3]

1 *Colegio de Postgraduados, Posgrado en Fitosanidad, Entomología y Acarología, Montecillo, Texcoco, Estado de México, Mexico* **2** *Canadian National Collection of Insects, Ottawa, Ontario, Canada* **3** *Department of Entomology, College Station, Texas A&M University, Texas, USA*

Corresponding author: J. Refugio Lomeli-Flores (jrlomelif@hotmail.com)

Academic editor: J. M. Jasso-Martínez | Received 3 October 2024 | Accepted 6 December 2024 | Published 5 March 2025

https://zoobank.org/42431DAD-708B-4396-B9F0-DB6BAABF573E

Citation: Rodríguez-Rodríguez SE, Fernández-Triana JL, Lomeli-Flores JR, Martínez-Núñez M, Rodríguez-Leyva E, González-Hernández H, Bernal JS (2025) *Apanteles piceotrichosus* Blanchard (Hymenoptera, Braconidae), first record from México, new molecular and biological data. Journal of Hymenoptera Research 98: 339–353. https://doi.org/10.3897/jhr.98.138425

Abstract

Between 1994 and 1995, *Cotesia vestalis* (Haliday 1834) (Hymenoptera, Braconidae) was introduced from the USA to Mexico to set up a biological control program for *Plutella xylostella*; nevertheless, its field establishment was never confirmed. From 2020 to 2024, a braconid parasitoid on *P. xylostella* larvae was collected in broccoli in Guanajuato state, the main brassicas producer in Mexico. Those collections had initially been linked to the former releases, but this work corroborated that *C. vestalis* has not been recovered in Guanajuato, but rather an unidentified species of another braconid was confirmed. The objective of this work was to identify it with classical and molecular taxonomy, and to describe its basic biology under laboratory conditions. Morphological identification was performed using the descriptions of Blanchard (1994) and molecular analysis was performed using the 16S, 28S and COI markers. The parasitoid was identified as *Apanteles piceotrichosus* Blanchard, this species has been previously reported only from Argentina, Chile and Brazil, so it represents the first record for Mexico and its gene sequence is reported for the first time. *A. piceotrichosus* is a solitary koinobiont endoparasitoid of *P. xylostella* larvae. The egg is hymenopteriform with an incubation period of 0.92 d; larval and pupal stages lasted 5.86 ± 0.01 d, and 5.65 ± 0.02 d, respectively. Overall, there were 12.43 ± 0.01 d from egg to adult emergence. With honey availability, females and males lived 16.08 ± 0.19 and 17.58 ± 0.54 d, respectively.

Copyright *Susana E. Rodríguez-Rodríguez et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Keywords
Biological control, Diamondback moth, koinobiont parasitoid, *Plutella xylostella*

Introduction

Plutella xylostella (L.) (Lepidoptera: Plutellidae), or diamondback moth (DBM), is an insect of Mediterranean origin but currently, a cosmopolitan pest of brassicas, and it is considered one of the most important pests in the world due to the damage it causes in those vegetables (Talekar and Shelton 1993; Zalucki et al. 2012; Furlong et al. 2013). The Integrated Pest Management (IPM) is the appropriate strategy to control it, but to achieve this approach it is indispensable to consider the contribution of natural enemies in each agroecosystem where IPM proposals must be made (Talekar and Shelton 1993; Bommarco et al. 2011; Bujanos-Muñiz et al. 2013; Karimzadeh and Besharatnejad 2020). Until now, more than 135 species of *P. xylostella* parasitoids have been identified (Delvare 2002; Sarfraz et al. 2005), and three genera of parasitoid wasps are considered the most important in some regions around the world: *Diadegma*, *Apanteles* and *Microplitis* (Lim 1986).

In North America, *Diadegma insulare* (Cresson) (Hymenoptera, Ichneumonidae) is the most widely distributed and abundant parasitoid of *P. xylostella* (Mitchell et al. 1997; Martínez-Castillo et al. 2002; Munir et al. 2015; Mason et al. 2022). In Canada, this parasitoid is distributed in all provinces, and together with other species of the parasitoid complex, parasitism levels of 3% to 17.6% were recorded (Mason et al. 2022). In the Lower Rio Grande Valley of Texas, USA, this parasitoid registered above 98% of the parasitoids reared from field-collected host larvae (Legaspi et al. 2000). In Central Mexico there are also important parasitism levels of *D. insulare*. For example, in Querétaro, the natural parasitism of *D. insulare* was 42, 44 and 45% in cabbage, cauliflower, and broccoli, respectively (Martínez-Castillo et al. 2002). In different regions of Guanajuato, the state with the highest brassicas production in Mexico, a few farmers are developing some conservation practices for natural enemies allowed to register up to 82% of parasitism on *P. xylostella*, mainly for *D. insulare* (Cid-Aguilar et al. 2023).

Despite *D. insulare* natural parasitism on *P. xylostella* in Guanajuato (Cid-Aguilar et al. 2023), more than 30 years ago some local farmers requested studies for the importation of *Cotesia vestalis* Haladay (Hymenoptera, Braconidae) previously knowns as *C. plutellae* (Kurdjumov), to increase the pest parasitism in the field (Chavez-Chavez 1991). After a technical evaluation, in 1994, several hundreds of *C. vestalis* were imported from the United States of America and released in Guanajuato, Mexico (Perales-Gutiérrez and Arredondo-Bernal 1999; Arredondo-Bernal and Rodríguez-Vélez 2020). After the introduction, some entomologists developed field sampling in Guanajuato but did not recover the imported parasitoid (Lomeli-Flores and Bernal 2022). Despite this situation, in Sinaloa, Cortez-Mondaca and Macías-Cervantes (2007) and in Durango, Mexico, García-Gutiérrez et al. (2009) reported parasitism on *P. xylostella* by *Cotesia* sp. without confirming the parasitoid species. From several dozen

field collections of *P. xylostella* in Guanajuato, from 2020 to 2024, a new parasitoid of Braconidae (*Apanteles* sp.) was identified. The parasitoid was more common than *D. insulare* in some Guanajuato localities (Lomeli-Flores and Bernal 2022). Because this *Apanteles* species seems to have an important role in natural control of *P. xylostella* in those agroecosystems, this work aimed to identify it with classical and molecular taxonomy, and to describe its basic biology under laboratory conditions.

Methods

Insect rearing

The *Apanteles* rearing was established from parasitized larvae of *P. xylostella* collected in broccoli at Dolores Hidalgo (21°10'53"N, 100°52'13"W), and San Luis de la Paz (21°09'51"N, 100°36'29"W), Guanajuato. In the laboratory, the parasitoid adults were offered for 24 h with 30 second and third-instar larvae of *P. xylostella* fed on broccoli leaves (*Brassica oleracea* var. Italica). After that, larvae were removed and they were fed on broccoli leaves and kept in a rearing chamber (25 ± 2 °C, 70% HR and 12:12 L:O photoperiod) at the Colegio de Postgraduados, Texcoco, Estado de Mexico. In the chamber (Felisa FE0147), we kept the parasitoid adults in plastic cages (10 cm Θ), usually five couples in each cage, and adults were provided with water and honey *ad libitum*. For basic biology observations of the parasitoid, we used *P. xylostella* larvae reared in broccoli plants (two months old), all assays were carried out under the environmental conditions already described.

Morphological identification

Morphological terms and measurements mostly follow Fernandez-Triana et al. (2014) and the Hymenoptera Anatomy Ontology Portal (http://portal.hymao.org/). The abbreviations T1 and T2 are used for metasomal mediotergites 1 and 2; and F15 refers to flagellomere 15. A previous revision of the genus *Apanteles* in Mesoamerica (Fernandez-Triana et al. 2014) and available, historical data on other species of *Apanteles* recorded as parasitoids of *P. xyllostella* such as Taxapad dataset (Yu et al. 2016) were consulted to identify the Mexican specimens.

We examined specimens from seven localities in Guanajuato (Fig. 1) (Suppl. material 1). One female and one male from León, Guanajuato (20°56'27.4"N, 101°37'31.2"W) were photographed to illustrate morphological details of the species (Fig. 2). Specimens are deposited in the National Collection of Insects, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City; the Insect Collection of the Colegio de Postgraduados (CEAM) (voucher code: CEAM-Hy-023), Texcoco, Estado de México; and the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Canada.

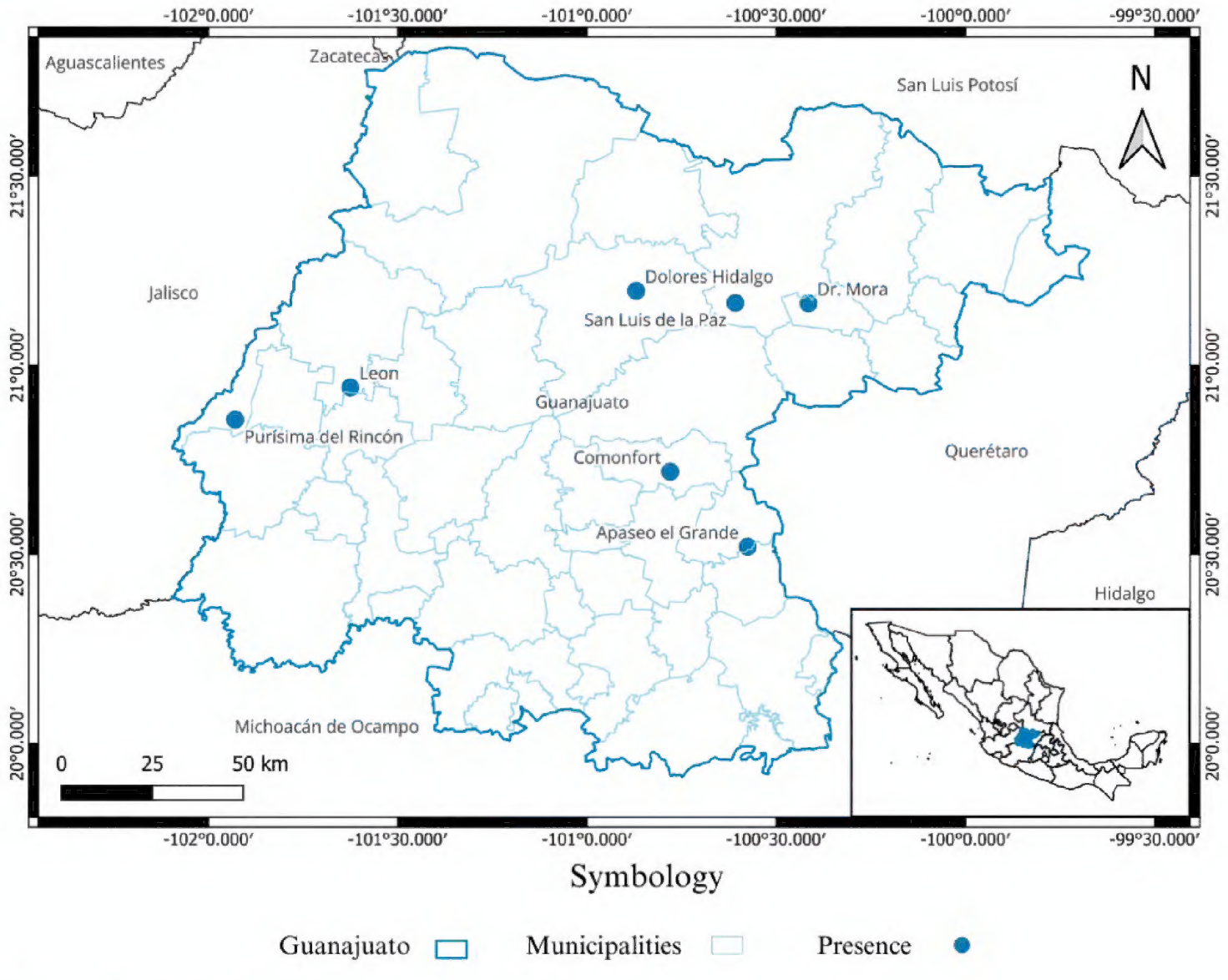

Figure 1. Distribution of *Apanteles piceotrichosus* in Guanajuato, Mexico.

Molecular identification, DNA extraction, amplification, and sequencing

An insect rearing was established from *Apanteles* specimens from Dolores Hidalgo and San Luis de la Paz. Ten *Apanteles* specimens (~15.0 mg in total) were randomly selected to DNA extraction using Quick-DNA Tissue/Insect Miniprep Kit (Zymo Research, USA). Briefly, specimens were placed into a ZR BashingBead™ Lysis Tube, and 750 µl BashingBead™ Buffer was added. The tubes containing each sample were processed at maximum speed in a FastPrep-24 tissue homogenizer (MPI Biomedicals, Santa Ana, CA, USA) for five min. Subsequently, samples were centrifuged at 10,000 × g for 1 min. The supernatant was transferred into a Zymo-Spin™ III-F Filter, centrifuged at 8,000 × g for 1 min, and the flow-through was recovered. 1,200 µl of Genomic Lysis Buffer was added to the filtrate in the Collection Tube, and the sample was perfectly homogenized. The mixture was placed in a Zymo-Spin™ IICR Column and centrifuged at 10,000 × g for 1 min. The flow through was discarded, and 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin™ IICR Column and centrifuged at 10,000 × g for 1 min. Later, we added 500 µl g-DNA Wash Buffer to the Zymo-Spin™ IICR Column and centrifuged at 10,000 × g for 1 min. After 50 µl of DNase/RNase-Free water was added directly to the column matrix, and DNA was eluted by centrifugation

at 10,000 × g for 30 s. Total DNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific) and stored at -20 °C until use. Two mitochondrial (16S and COI) and one nuclear marker (28S rRNA) were amplified using specific primers (Table 1).

PCR mixes had a total volume of 20.0 µl and contained 8.4 µl of ddH20, 4.0 µl of 5X Phusion™ HF Buffer, 1.0 µl of 10 µM of each primer, 0.4 µl of 10 mM dNTPs, 0.2 µl of Phusion™ High-Fidelity DNA Polymerase (2 U/µl), and 5 µl of DNA template. The polymerase chain reaction (PCR) conditions were: Initial denaturation for 30 s at 98 °C, followed by 35 cycles of 5 s denaturation at 98 °C, 30 s primer annealing at 53 °C for COI and 55 °C for 16S and 28S, 30 s initial extension at 72 °C and a final extension of 5 min at 72 °C, followed by indefinite hold at 4 °C.

The amplified PCR products of 451 bp, 487 bp, and 383 bp for 16S, 28S, and COI, respectively, were electrophoresed in 1.0% agarose gels stained with ethidium bromide (0.5 µg/µL) and visualized on an ultraviolet trans-illuminator (Gel Doc™ EZ System, Bio-Rad, USA).

The PCR products were purified with a Zymo DNA Clean and Concentrator kit (Zymo Research, Irvine, CA). Purified amplicons were sent to Unidad de Síntesis y Secuenciación de DNA-UNAM for Sanger sequencing (USSDNA, UNAM, México). All products were sequenced from both strands. One COI sequence, representative of the species, was deposited in BOLD (Barcoding of Life Data System, https://v4.boldsystems.org/index.php).

Table 1. Primers used for amplification and sequencing of molecular markers 16S, 28S, and COI.

Gene	Name	Sequence	Reference
16S	F: 16Sf f	CACCTGTTTATCAAAAACAT	Dowton and Austin (1994)
	R: 16S r	CTTATTCAACATCGAGGTC	Whitfield (1997)
28S	F: 28sf	AGAGAGAGTTCAAGAGTACGTG	Belshaw and Quicke (1997)
	R: 28sr	TTGGTCCGTGTTTCAAGACGGG	Campbell et al. (2000)
COI	F: C1-J-1859	GGAACTGGATGAACAGTATAT	Simon et al. (1994)
	R: C1-N-2191	CCAGGTAAAATTAAAATATAAACTTC	

Tree reconstruction of mtDNA COI sequences

The COI sequence of *A. piceotrichosus*, from a specimen reared from *P. xylostella* in Mexico in this study (BOLD Process ID: CEAM-Hy-023), now allows for recognition of the species using the BOLD database. The generated Cytochrome c oxidase subunit I sequences were analyzed with BLAST (Basic Local Alignment Search Tool) to identify available similar sequences in NCBI (National Centre for Biological Information), and use them for manual data curation utilizing BioEdit 7.2.6. (Hall 1999). Similar sequences were aligned using the ClustalW program integrated in MEGA X 7.0.26 (Kumar et al. 2018). Maximum likelihood analyses were performed based on the Tamura-Nei model (Tamura and Nei 1993) using the MEGA X software (Kumar

et al. 2018). The tree with the highest log probability (-7297.69) was developed. The initial trees for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with the highest log-likelihood value. This analysis involved 95 nucleotide sequences; included codon positions (for amino acid sequences), and No coding (for DNA sequences) to allow for a thorough comparison at both levels. There was a total of 322 positions in the final data set; the evolutionary analyzes were carried out in MEGA X.

Cytochrome c oxidase subunit I sequence of *Agathirsia davidi* (GenBank Accession number MF098373.1) was used as an out-group in the phylogenetic study.

Phylogenetic analysis: Concatenated 16s-28s-COI

The evolutionary history was inferred using the Maximum Likelihood method and the Tamura-Nei model. The tree with the highest logarithmic probability (-1951.31) was constructed. The initial trees for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with the highest log-likelihood value. This analysis involved eight nucleotide sequences. There was a total of 865 positions in the final data set. 16S ribosomal RNA, 28S ribosomal RNA, and Cytochrome c oxidase subunit I sequences of *Alphomelon* sp. (GenBank Accession numbers AF102752.1, AF102732.1, and AF102707.1 respectively) were used as out-group in the phylogenetic study. Evolutionary analyses were performed in MEGA X.

Biology and developmental time

After some preliminary laboratory observations, we detected successful parasitism only in second- and third-instar *P. xylostella* larvae. The experimental units for this assay were Petri dishes (5.5 cm Θ) with a small perforation (1.0 cm Θ) covered with organza fabric for ventilation. In each arena, 10 second-instar larvae of *P. xylostella* were exposed for 5 min to an *Apanteles* female 2–5 days old with oviposition experience. Immediately after detecting that the parasitoid showed oviposition behavior on a larva, using an entomological brush (# 0), the larva was individualized in a new Petri dish (3 cm Θ) where a broccoli leaf disk was placed for its feeding. Ten repetitions were performed, the broccoli leaf disk was replaced every 24 h for each larva until the emergence of the parasitoid. All the experimental units were incubated in a bioclimatic chamber at 25 ± 2 °C, 70% HR and 12:12 L:O photoperiod.

To determine the incubation period, we dissected 10 larvae at 18, 24 and 30 h (n = 30) after exposure to parasitoids. In addition, 70 larvae were selected and observed every 24 h to determine the duration of larval and pupal stages. Duration of the larval period was determined considering data from the previous assay (incubation time), until the formation of the cocoon. The pupal period was considered from the cocoon to the emergence of the adult. Finally, the longevity of 24 females and 24 males was recorded using Petri dishes (3.5 cm Θ) with a perforation (1.5 cm Θ) covered with

organza fabric for ventilation. Each sex and group of adults was offered a solution of honey and water or simply water. The description of each stage of development (egg, larva, and pupa) was made from photographs taken with a Digital camera Canon EOS 5D adapted to a light microscope Carl Zeiss stereo discovery UV20 and consulting Clausen (1940) and Stehr (1987) literature.

Results and discussion

Morphological identification

The identification was done by JFT, who is the author of the 2014 monograph on the 205 species of Mesoamerican *Apanteles* (and has also described *Apanteles* species from other regions of the world, e.g,, Canada, USA, Europe). The work of JFT carefully considered all of the details provided in the original description, which happened to be unusually detailed and clear, that way helping to contrast the information versus the Mexican specimens at hand. Furthermore, the specimens from Argentina were also reared from the same host species, which happens to be a common and ubiquitous pest distributed worldwide and therefore the parasitoid could have followed the pest (or be present in Mexico already, both hypothesis are reasonable and plausible, although that was not tested, therefore that speculation is not included in this paper). Although one can never be 100% sure about the accuracy of any taxonomic determination, the fact that morphological characters were all strongly concordant, the host species was the same and the relative experience of the taxonomist involved with this genus of parasitoid wasp, the assumption of the species being the same is very reasonable and, we would argue, sufficiently supported by evidence (morphology and biology).

The Mexican specimens were identified as *Apanteles piceotrichosus* Blanchard, 1947. Until now this species had only been recorded from the southern half of South America: Argentina (Buenos Aires) (Blanchard 1947), Brazil (Rio Grande do Sul) (Ferronatto and Becker 1984; Goncalves and Mare 2005), and Chile (near Santiago de Chile) (Muriel and Grez 2003), all known localities falling below 30°S. This is the first report of *A. piceotrichosus* in Mexico (at around 20°N, see Suppl. material 1) and indeed constitutes the first record for North America.

The Mexican specimens we studied match almost perfectly with the original description of *A. piceotrichosus* (Blanchard 1947: 18–20), which included several drawings illustrating the tip of the antenna, scutellum, metascutellum, propodeum, metasomal tergites 1 and 2, and half of the fore wing; there are also extensive body measurements and detailed accounts of body color and sculpture.

The relatively recent revision of the genus *Apanteles* in Mesoamerica (Fernandez-Triana et al. 2014) did not include *A. piceotrichosus* among the 205 species dealt with there because at the time *piceotrichosus* was considered to be restricted to South America. In the key to species-group provided in that paper, specimens of *A. piceotrichosus* will run up to the second half of couplet 49, which includes the *Apanteles adrianachavarriae* species-group. It will reach up that point based on the following mor-

phological traits: relatively short ovipositor sheaths, pterostigma mostly transparent or white but with thin brown borders, tegula of a different color than humeral complex, T2 transverse and relatively narrow, pleated hypopygium and body color, especially legs. The *adrianachavarriae* species-group includes nine species, but all of them can be easily separated from *piceotrichosus* because they have T1 broad and parallel-sided (T1 strongly narrowing towards the posterior margin on posterior half of tergite in *piceotrichosus*) and ovipositor sheaths longer than metatibia (sheaths length 0.8–0.9× metatibia length in *piceotrichosus*). There are also differences in leg coloration, with *piceotrichosus* usually having darker coloration, especially on hind legs, than all the species in the *adrianachavarriae* group. Apart from the keys provided in Fernandez-Triana et al. (2014), it is important to diagnose *A. piceotrichosus* from three other species of *Apanteles* previously recorded as parasitoids of *P. xyllostella* within the New World, due to the wide distribution of the lepidopteran in the continent.

a) *Apanteles alexanderi* Brèthes, 1922. It has F15 cubic to subcubic, i.e., F15 length around 1.0 × F15 width (F15 length 1.2–1.4 × F15 width in *A. piceotrichosus*, Fig. 2C); T1 mostly parallel-sided (T1 rather strongly narrowing on posterior third in *A. piceotrichosus*, Fig. 2I); T2 with some punctures, especially on posterior half (T2 almost entirely smooth, at most with few scattered and weak punctures on posterior margin in *A. piceotrichosus*, Fig. 2I); T2 subrectangular, with lateral margins mostly parallel, only slightly widening towards posterior margin (T2 trapezoidal to subtriangular, with lateral margins strongly divergent towards posterior margin in *A. piceotrichosus*, Fig. 2); pro- and mesofemora yellow (pro- and mesofemora black in *A. piceotrichosus*, Fig. 2A, B); and ovipositor sheaths 1.4× metatibia length (ovipositor sheaths around same length than metatibia length in *A. piceotrichosus*, Fig. 2A). Also, the record of *A. alexanderi* as parasitoid of *Plutella xyllostella* has been recently questioned by Martinez et al. (2012).

b) *Apanteles epinotiae* Viereck, 1912. It has F15 cubic to subcubic, i.e., F15 length around 1.0 × F15 width (F15 length 1.2–1.4 × F15 width in *A. piceotrichosus*, Fig. 2C); T1 mostly parallel-sided (T1 rather strongly narrowing on posterior third in *A. piceotrichosus*, Fig. 2I); propodeum with complete and strong areola (propodeum with relatively weak areola, only marked on posterior half in *A. piceotrichosus*, Fig. 2F, I); most veins transparent, with only very few white and the outline of pterostigma and part of vein R1 very light brown (darker vein coloration in *A. piceotrichosus*, Fig. 2A, B)

c) *Apanteles sodalis* (Haliday, 1834). It has pterostigma almost entirely brown, with a pale spot at base (pterostigma mostly pale with only thin brown margins in *A. piceotrichosus*, Fig. 2A, B); T1 parallel-sided (T1 rather strongly narrowing on posterior third in *A. piceotrichosus*, Fig. 2I); T2 sculptured with rather strong striae (T2 almost entirely smooth, at most with few scattered and weak punctures on posterior margin in *A. piceotrichosus*, Fig. 2I); T2 subrectangular, with lateral margins mostly parallel-sided (T2 trapezoidal to subtriangular, with lateral margins strongly divergent towards posterior margin in *A. piceotrichosus*, Fig. 2I); and tegula brown (tegula paler in *A. piceotrichosus* Fig. 2F, G).

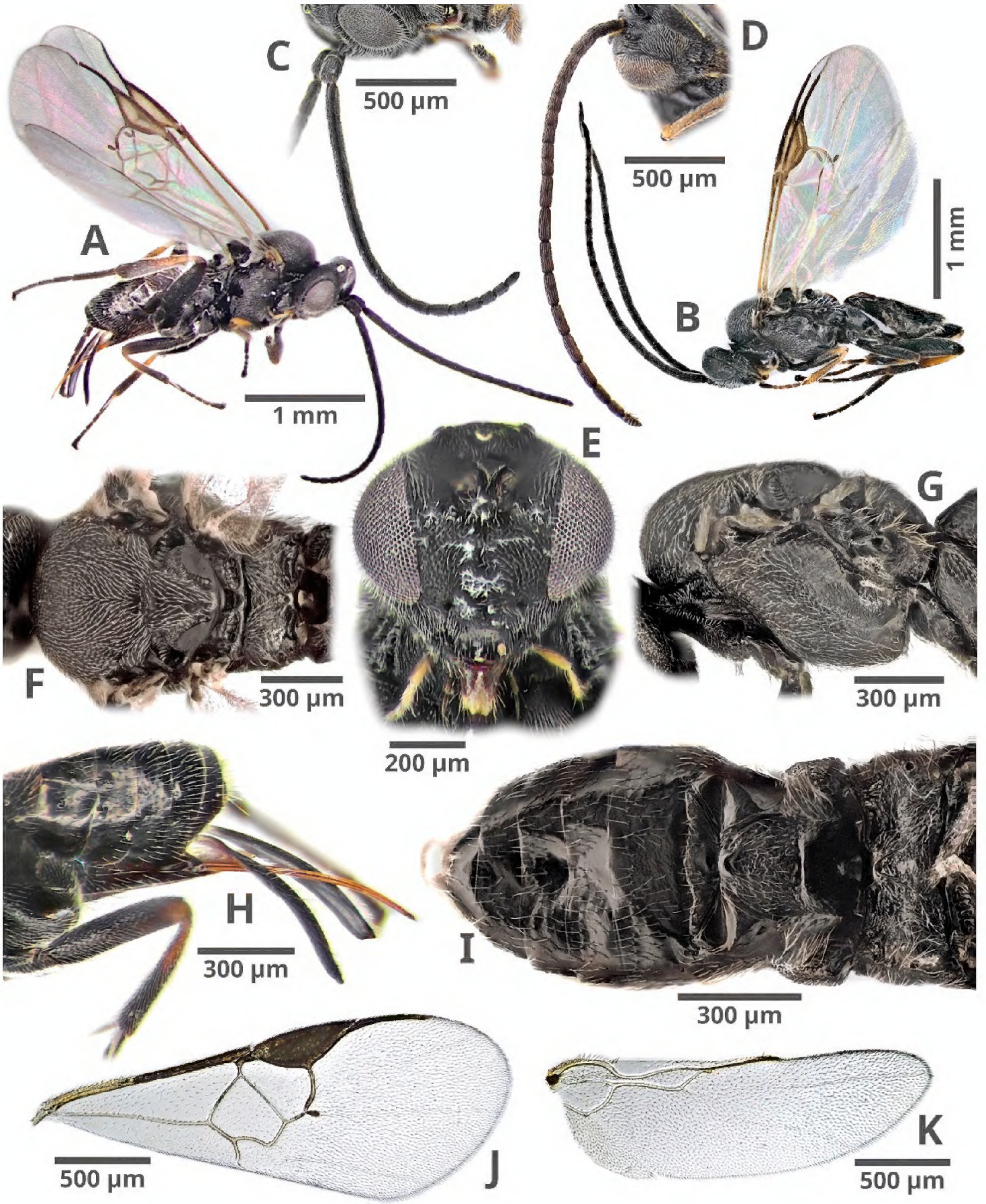

Figure 2. *Apanteles piceotrichosus* **A** habitus lateral female **B** habitus lateral male **C** antenna female **D** antenna male **E** head female, frontal **F, G** mesosoma female, dorsal and lateral **H** metasoma and ovipositor sheaths **I** propodeum and metasoma female, dorsal **J, K** fore, and hind wings.

In contrast to those three species, *A. piceotrichosus* has longer F15 (F15 length 1.2–1.4 × F15 width); darker legs (profemur brown, mesofemur dark brown to black); shorter ovipositor sheaths (slightly less to around same length than metatibia); and propodeum with relatively weak areola, only marked on posterior half.

Molecular identification

COI has been the most commonly and widely used molecular marker for species identification in Microgastrinae, with high success rate to distinguish and separate species (e.g., Smith et al. 2013; Fernandez-Triana et al. 2020). Inclusion of other genes would be useful to explore phylogenetic relationships at a higher level, however in this paper we aimed to see if the DNA barcodes obtained were sufficient to characterize *Apanteles piceotrichosus*. The COI sequence CEAM-Hy-023, deposited in BOLD, allows for unambiguous recognition of the species based on DNA barcoding, as this sequence is 5.91–6.09% different (38–40 base pairs of difference) from the closest species in the BOLD database, *Apanteles javiercontrerasi* Fernandez-Triana (2014) and *A. carlosguadamuzi* Fernandez-Triana (2014). The molecular analysis confirms that the parasitoid collected on *P. xylostella* in Guanajuato corresponds to the genus *Apanteles*. Furthermore, the phylogenetic trees suggest that it is a species close to *G. merope* and *Apanteles melpomene* Nixon, 1965 (Suppl. material 2) The first species was recently changed to the genus *Glyptapanteles* by Fernández-Triana et al. (2020) and is only registered in Malaysia. On the other hand, *A. melpomene* has also been recorded only from Malaysia.

Biology and developmental time

Apanteles piceotrichosus is a solitary koinobiont endoparasitoid of *P. xylostella* larvae, we corroborated that it can parasitize second and third-instar and we never recovered parasitoids of fourth-instar larva. We believe that when there was parasitism in some first-instar larvae they failed to develop; right now some assays are being developed to confirm which instar is preferred for parasitism. The egg is typically hymenopteriform, translucent, elongated, and has a short pedicel. The incubation period was 0.92 ± 0.00 d. The first instar larva has a pair of jaws and what appears to be the anterior region of the head capsule is wider than the rest of the body. From the second instar, the formation of an anal vesicle can be seen that increased in size in more developed larval stages, which gives them the name of vesiculate type, the vesicle is the evagination of the proctodeum (Poinar and Thomas 1984). This species probably goes through five larval instars, although the duration of each one is not yet known. The larval stage lasted 5.86 ± 0.01 d. When a *P. xylostella* parasitized larva began to spin its cocoon to pupate, the parasitoid larva pierced the integument and emerged from the *P. xylostella* larva, completely consumed its host fluids, and then it spun its cocoon to pupate. The pupa is exarate and presents similar characteristics to the adult, with a yellowish coloration, finally the recently emerged adult is completely black. The pupal period lasted 5.65 ± 0.02 d. Mean developmental time from egg to adult was 12.43 ± 0.01 d. Male and female parasitoids provided with honey solution lived longer, 17.58 ± 0.54 and 16.08 ± 0.19 d, respectively, than those with only water (2.42 ± 0.51 and 3.08 ± 0.08 d, respectively).

The developmental time was similar to *Apanteles machaeralis* with 12.63 d (Clement and David 1990), and *Apanteles syleptae* F. which duration ranged from 11 to 14 d (El-Sherif and Kaschef 1977). The studies that have been carried out with the species

have only determined that it prefers to parasitize the first two larval instars of its host and the parasitism becomes 17% (Ferronatto and Becker 1984). Its biological characterization is limited (Muriel and Grez 2003; Goncalves and Mare 2005), and more work is needed to determine its role as a natural enemy of *P. xylostella* in the field.

Acknowledgements

We thank Jorge M. Valdez-Carrasco for his invaluable aid in capture and processing of images. We are also grateful to Comité Estatal de Sanidad Vegetal del Estado de Guanajuato (CESAVEG), and Secretaría de Agricultura y Desarrollo Rural del Estado de Guanajuato for providing financial support to improve control of *P. xylostella* in Guanajuato. To the National Council of Humanities, Sciences, Technologies, and Innovation (CONAHCYT), Mexico, for the award of a doctoral scholarship to the first author (CVU number 662879). JLFT was supported by Agriculture and Agri-Food Canada Project J-002276 Systematics of beneficial arthropods in support of resilient agroecosystems.

References

Arredondo-Bernal HC, Rodríguez-Vélez B (2020) Biological Control in Mexico. In: van Lenteren, JC, Bueno VHP, Luna MG, Colmenarez Y (Eds) Biological Control in Latin America and the Caribbean: Its Rich History and Bright Future. CAB International, 308–335. https://doi.org/10.1079/9781789242430.0308

Belshaw R, Quicke DLV (1997) A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). Molecular Phylogenetics and Evolution 7(3): 281–293. https://doi.org/10.1006/mpev.1996.0400

Blanchard EE (1947) Descripciones y anotaciones de microgastrinos argentinos (Hymenoptera). Arthropoda 1(1): 6–22.

Bommarco R, Miranda F, Bylund H, Björkman C (2011) Insecticides suppress natural enemies and increase pest damage in cabbage. Journal of Economic Entomology 104(3): 782–791. https://doi.org/10.1603/EC10444

Bujanos-Muñiz R, Marín-Jarillo A, Díaz-Espino LF, Gámez-Vázquez AJ, Ávila-Perches MA, Herrera-Vega R, Dorantes-González JRA, Gámez-Vázquez FP (2013) Manejo Integrado de la Palomilla Dorso de Diamante *Plutella xylostella* (L.) en la región del Bajío, México. Folleto técnico número 27, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP).

Campbell B, Heraty J, Rasplus JY, Chan K, Steffen-Campbell J, Babcock C (2000) Molecular systematics of the Chalcidoidea using 28S-D2 rDNA. In: Hymenoptera evolution, biodiversity and biological control. CSIRO Publishing (Collingwood, Australia), 59–73.

Chávez-Chávez MT (1991) Primeras pruebas de control biológico de *Plutella xylostella* con el braconido *Cotesia plutellae*. Resúmenes del XIV Congreso Nacional de Control Biológico. Universidad Autónoma Agrícola "Antonio Narro". Saltillo, Coahuila.

Cid-Aguilar SM, Lomeli-Flores JR, Rodríguez-Leyva E, Tamayo-Mejía F (2023) Uso de fructosa y plantas con flores para fomentar el control biológico de *Plutella xylostella*. Revista Fitotecnia Mexicana 46(1): 21–31. https://doi.org/10.35196/rfm.2023.1.21

Clausen CP (1940) Entomophagous Insects. McGraw-Hill Book Company (New York and London), 1–688.

Clement P, David BV (1990) Biology of *Apanteles machaeralis* Wilkison (Hymenoptera: Braconidae) a parasite of *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae). Proceedings of the Indian National Science Academy (Anim. Sci.) 99(5): 353–362. https://doi.org/10.1007/BF03191867

Cortez-Mondaca E, Macías-Cervantes J (2007) Parasitismo natural de la palomilla dorso de diamante *Plutella xylostella* L. en canola (*Brassica napus* L.), en el Norte de Sinaloa, México. Agrociencia 41: 347–354.

Dowton M, Austin AD (1994) Molecular phylogeny of the insect order Hymenoptera: Apocritan relationships. Proceedings of the National Academy of Sciences of the United States of America 91(21): 9911–9915. https://doi.org/10.1073/pnas.91.21.9911

Delvare G (2002) The taxonomic status and role of Hymenoptera in biological control of DBM, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). In: Kirk AA, Bordat D (Eds) Improving biocontrol of *Plutella xylostella*. Proceedings of the International Symposium (Montpellier, France), October 2002. CIRAD, 17–49.

El-Sherif LS, Kaschef AH (1977) Morphological and biological studies on *Apanteles syleptae* F. (Hymen., Braconidae), recovered from the jasminium moth, *Palpita unionalis* Hb. Z. ang. Ent. 84: 419–424. https://doi.org/10.1111/j.1439-0418.1977.tb04304.x

Fernandez-Triana J, Shaw MR, Boudreault C, Beaudin M, Broad GR (2020) Annotated and illustrated world checklist of Microgastrinae parasitoid wasps (Hymenoptera, Braconidae). ZooKeys 920: 1–1089. https://doi.org/10.3897/zookeys.920.39128

Fernández-Triana JL, Whitfield JB, Rodriguez JJ, Smith MA, Janzen DH, Hallwachs W, Hajibabaei M, Burns JM, Solis MA, Brown J, Cardinal S, Goulet H, Hebert PDN (2014) Review of *Apanteles* sensu stricto (Hymenoptera, Braconidae, Microgastrinae) from Area de Conservación Guanacaste, northwestern Costa Rica, with keys to all described species from Mesoamerica. ZooKeys 383: 1–565. https://doi.org/10.3897/zookeys.383.6418

Ferronatto EM, Becker M (1984) Abundância e complexo de parasitoides de *Plutella xylostella* Linnaeus, 1758) (Lepidoptera: Plutellidae) em *Brassica oleracea* L. var. acephala DC. Anais da Sociedade Entomologica do Brasil 13(2): 261–278. https://doi.org/10.37486/0301-8059.v13i2.350

Furlong MJ, Wright DJ, Dosdall LM (2013) Diamondback moth ecology and management: problems, progress, and prospects. Annual Review of Entomology 58: 517–541. https://doi.org/10.1146/annurev-ento-120811-153605

García-Gutiérrez C, González-Maldonado MB, Chaírez Hernández I, Bautista-Martínez N (2009) Parasitismo natural de las principales plagas del repollo en Nombre de Dios, Durango. Southwestern Entomologist 34(2): 193–195. https://doi.org/10.3958/059.034.0210

Gonçalves RR, Mare RAD (2005) Biologia da traça das crucíferas, *Plutella xylostella* Linnaeus (Lepidoptera, Yponomeutidae), sob condições controladas de temperatura, e parasitóides associados: Part III. Estudo sobre a biologia de *Apanteles piceotrichosus* (Blanchard)

(Hymenoptera, Braconidae): análise do efeito de endocruzamento. Revista Brasileira de Zoologia 22: 806–809. https://doi.org/10.1590/S0101-81752005000300045

Hall TA (1999) BioEdit: A User-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

HAOP [The Hymenoptera Anatomy Ontology Portal] (2024) The Hymenoptera Glossary: Hymenoptera Anatomy Consortium. Accessed on Mon Jun 17 16:39:03 -0500 2024. http://glossary.hymao.org

Karimzadeh J, Besharatnejad MH (2020) Ecological control of *Plutella xylostella* (Lepidoptera, Plutellidae) using trap cropping and Bt applications. Archives Phytopathology and Plant Protection. https://doi.org/10.1080/03235408.2019.1707930

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetic Analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Lim GS (1986) Biological control of diamondback moth. In: Talekar NS, Griggs TD (Eds) Diamondback Moth Management: Proceedings of the First International Workshop. Asian Vegetable Research and Development Center (Shanhua, Taiwan), 159–171.

Legaspi Jr BC, Liu TX, Sparks Jr A (2000) Occurrence of the diamondback moth and its parasitoids in the Lower Rio Grande Valley of Texas. Subtropical Plant Science 52: 47–51.

Lomeli-Flores JR, Bernal J (2022) Nuevas alternativas de control de la palomilla dorso de diamante. Informe técnico. Colegio de Postgraduados y Secretaría de Desarrollo Agroalimentario y Rural del Estado de Guanajuato, Convenio DS/SDCA/DGA/DSV/08/2021.

Martínez-Castillo M, Leyva JL, Cibrian-Tovar J, Bujanos-Muñiz R (2002) Parasitoid diversity and impact on populations of the diamondbak moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), on *Brassica* crops in central Mexico. BioControl 47: 23–31. https://doi.org/10.1023/A:1014423106261

Martínez JJ, Berta C, Varone L, Logarzo G, Zamudio P, Zaldívar-Riverón A, Aguilar-Velasco, R G (2012) DNA barcoding and morphological identification of Argentine species of *Apanteles* (Hymenoptera: Braconidae), parasitoids of cactus-feeding moths (Lepidoptera: Pyralidae: Phycitinae), with description of a new species. Invertebrate Systematics 26: 435–444. https://doi.org/10.1071/IS12060

Mason PG, Dancau T, Abram PK, Noronha C, Dixon PL, Parsons CK, Bahar MH, Bennett AMR, Fernández-Triana J, Brauner AM, Clarke P, Thiessen J, Gillespie DR, Haye T (2022) The parasitoid complex of diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), in Canada: impact and status. The Canadian Entomologist 154(e12): 1–26. https://doi.org/10.4039/tce.2021.51

Mitchell ER, Hu GY, Okine JS (1997) Diamondback moth (Lepidoptera: Plutellidae) infestation and parasitism by *Diadegma insulare* (Hymenoptera: Ichneumonidae) in collards and adjacent cabbage fields. Florida Entomologist 80(1): 54–62. https://doi.org/10.2307/3495976

Munir S, Dosdall LM, O'Donovan JT (2015) Evolutionary ecology of diamondback moth, *Plutella xylostella* (L.) and *Diadegma insulare* (Cresson) in North America: A Review. Annual Research and Review in Biology 5(3): 189–206. https://doi.org/10.9734/ARRB/2015/11834

Muriel SB, Grez AA (2003) Abundancia y parasitismo de *Plutella xylostella* L. (Lepidoptera: Plutellidae) en parches de *Brassica oleracea* con diferente forma y vegetación circundante. Actualidades Biológicas 25(79): 1–8. https://doi.org/10.17533/udea.acbi.329489

Perales-Gutiérrez MA, Arredondo-Bernal HC (1999) *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae). Ficha técnica CB-13. Centro Nacional de Referencia de Control Biológico, Tecomán, Colima, México.

Poinar GO, Thomas GM. 1984. Immature stages of insect endoparasites. In: Laboratory guide to insect pathogens and parasites. Springer (Boston), 281–325. https://doi.org/10.1007/978-1-4684-8544-8_8

Salas-Araiza MD, Salazar-Solis E (1998) Parasitismo natural de lepidópteros plagas de brócoli en el Bajio, México. Manejo Integrado de Plagas (Costa Rica) 50: 34–41.

Sarfraz M, Keddie AB, Dosdall LM (2005) Biological control of the diamondback moth, *Plutella xylostella*: A review. Biocontrol Science and Technology 15(8): 763–789. https://doi.org/10.1080/09583150500136956

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting and phylogenetic utility of mitocondrial gene sequences and a compilation of conserved polymerase chain reaction "primers". Annals of the Entomological Society of America 87(6): 651–670. https://doi.org/10.1093/aesa/87.6.651

Smith MA, Fernandez-Triana J, Eveleigh E, Gómez J, Guclu C, Hallwachs W, Hebert PDN, Hrcek J, Huber JT, Janzen DH, Mason PG, Miller SE, Quicke D, Rodriguez JJ, Rougerie R, Shaw MR, Varkonyi G, Ward D, Whitfield JB, Zaldívar-Riverón A (2013) DNA barcoding and the taxonomy of Microgastrinae wasps (Hymenoptera, Braconidae): impacts after eight years and nearly 20,000 sequences. Molecular Ecology Resources 13: 168–176. https://doi.org/10.1111/1755-0998.12038

Stehr FG (1987) Immature Insects, Volumen 1. Kendall/Hunt Publishing Company (United States of America), 754 pp.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology Evolution 10(3): 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Talekar NS, Shelton AM (1993) Biology, ecology, and management of the diamondback moth. Annual Review of Entomology 38: 275–301. https://doi.org/10.1146/annurev.en.38.010193.001423

Whitfield JB (1997) Molecular and morphological data suggest a single origin of the polydnaviruses among Braconid wasps. Naturwissenschaften 84: 502–507. https://doi.org/10.1007/s001140050434

Yu DSK, van Achterberg C, Horstmann K (2016) Taxapad 2016, Ichneumonoidea 2015. Database on flash-drive. Nepean, Ontario. http://www.taxapad.com

Zalucki MP, Shabbir A, Silva R, Adamson D, Shu-Sheng L, Furlong MJ (2012) Estimating the Economic Cost of One of the World's Major Insect Pests, *Plutella xylostella* (Lepidoptera: Plutellidae): Just how long is a piece of string?. Journal of Economic Entomology 105(4): 1115–1129. https://doi.org/10.1603/EC12107

Supplementary material 1

Geographical coordinates of *Apanteles piceotrichosus* (Hymenoptera, Braconidae) collection sites in Guanajuato, Mexico

Authors: Susana E. Rodríguez-Rodríguez, José L. Fernández-Triana, J. Refugio Lomeli-Flores, Marcelino Martínez-Núñez, Esteban Rodríguez-Leyva, Héctor González-Hernández, Julio S. Bernal

Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/jhr.98.138425.suppl1

Supplementary material 2

Phylogenetic tree

Authors: Susana E. Rodríguez-Rodríguez, José L. Fernández-Triana, J. Refugio Lomeli-Flores, Marcelino Martínez-Núñez, Esteban Rodríguez-Leyva, Héctor González-Hernández, Julio S. Bernal

Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/jhr.98.138425.suppl2